AN ANALYSIS OF CERTAIN CASES OF INTRA-SPECIFIC STERILITY

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INTRODUCTION

In the course of the experiments upon *Drosophila melanogaster* Meigen (*ampelophila* Loew) carried on at COLUMBIA UNIVERSITY, a number of mutant stocks have been obtained in which either the male or female produces few or no young. On account of both its economic and its scientific interest the general phenomenon of sterility has been the subject of investigation and discussion for a period of many years and the mass of facts which has accumulated has served to demonstrate the complexity of the problem. One of the principal conclusions from the evidence is, that the various phases of sterility are undoubtedly to be referred to diverse causes. It is a widespread belief that one of the commonest causes is the

[1] From the Zoölogical Laboratory of COLUMBIA UNIVERSITY, New York.

process of inbreeding and in several cases this idea has been supported by experimental evidence, but in the present instance, such a connection is not involved since in every race investigated the attribute in question appears in conjunction with specific somatic characters and follows the distribution of the mutant gene responsible for them, regardless of whether the stock has been inbred or not. In fact, on account of the sterility involved, out-crossing has usually been a necessity for the continuation of the strain. With the Mendelian theory as an instrument of investigation, it has been possible to demonstrate the existence of definite factors which are responsible for the degree of sterility shown. One such factor has already been indicated in Drosophila by the work of HYDE (1911) and two in the fowl by PEARL (1912). With the possibility of other similar discoveries in view, a brief inquiry into the nature and behavior of certain sterile stocks of Drosophila was begun.

The mutant races examined were seven in number and included rudimentary wings, fused wings, cleft wings, morula eyes, reduced bristle, dwarf body, and dibro. These characters are due to recessive genes widely distributed in the germ plasm, as the first three have been located in the first or X chromosome, morula and reduced bristle in the second, and dwarf in the third. Dibro has not yet been placed. In most of these strains it is the female which is markedly unproductive, but in cleft, however, the male is sterile, and since the character is sex-linked it has been impossible to obtain cleft females. In dibro both sexes have so far proved completely sterile, and a large enough number of individuals has not yet been tested to make it certain that fertile ones are not produced.

FUSED

a. *Sterility in the fused stock*

The gene representing fused has been located at 59.5 in the X chromosome. Its chief effect upon the soma is to produce a more or less complete fusion of the third and fourth wing veins and a striking degree of infertility in the female. Stock is kept up by mating the male with heterozygous females and thus obtaining normal males and normal females and fused males and fused females in practically a 1 : 1 : 1 : 1 ratio. Table 1 summarizes the results from ten such crosses. Two exceptional classes, eosin-eyed females and red males, due to non-disjunction, may be omitted and the eye colors, eosin, white and red, disregarded in the comparison of the fused and normal offspring. The total number of

TABLE I

Normal ♀ heterozygous for fused by red fused ♂.

No. of culture	Normal ♀♀	Fused ♀♀	Eosin ♀♀	Eosin ♂♂	White ♂♂	Eosin fused ♂♂	White fused ♂♂	Red fused ♂♂	Total
L	95	76	13	43	34	31	30	6	328
K	94	56	6	42	37	16	30	8	289
B	108	123	14	71	35	54	69	7	481
F	110	76	8	52	28	36	55	8	373
D	95	122		56	29	31	76		409
A	87	82		45	29	32	43		318
H	102	121		52	38	54	49		416
2	47	39	4	29	17	20	30	1	187
3	53	45	10	32	18	17	25	3	203
4	53	46	4	38	23	20	43		227
	844	786	59	460	288	311	450	33	
Total	844	786		748		761			

flies per bottle is slightly below normal but this may be accounted for in part by the fact that the temperature was below the optimum and because no effort was made to continue the counts up to the very end of the hatching period, nor were the females transferred to fresh bottles of food in order to obtain the maximum output of eggs. However, even allowing for these circumstances, we are probably justified in concluding that the heterozygous female is slightly affected in the direction of sterility by the presence in her chromosomal constitution of the factor for fused. The totals obtained for the four classes are: normal females 844, fused females 786, normal males 748, fused males 761. It is a general rule in any cross in Drosophila that fewer males than females are produced unless counts are continued until the bottle runs out so that the slight differences in regard to the sexes observed here may not be of particular significance. Comparing the total number of normal individuals with the total number of fused (exclusive of the two exceptional classes) we find there are 1592 to 1547,—figures which do not suggest any noticeable difficulty in the formation of the fused class from the heterozygous type. The harmfulness of the fused gene, therefore, affects the entire output of gametes in the heterozygous female rather than any one variety.

The low fertility of the fused female seems to be partly due to the fact that the normal number of eggs is not produced. Examination of the ovaries shows that fewer eggs than usual mature and by means of

egg counts it was ascertained that the number laid is below normal. Table 2 gives the data from seven crosses showing the number of eggs laid and of flies hatched therefrom. The largest number of eggs deposited by any one female was 88, none of which produced an adult fly. In the bottles where the males used were from normal stock, some of the eggs had produced larvae before they were counted and transferred

TABLE 2

Showing the number of eggs laid and of flies hatched by fused females.

No. of culture	Parents ♀	Parents ♂	Eggs transferred	Larvae transferred	Adults produced
1	*fu*	*fu*	23	0	0
2	"	"	88	0	0
3	"	"	21	1	0
4	"	*N*	41	25	25
5	"	"	25	4	3
6	"	"	37	11	15
7	"	"	37	16	5
Total			272	57	48

to fresh food. The correspondence between the number of larvae produced previous to the transfer and of flies hatched, seemed to indicate that the eggs might have been injured by handling. To test this, fused females were mated with normal males and provided with food spread in a transparent layer upon glass slides so that the eggs when laid might be easily seen and counted without necessitating their removal. Every few days the eggs were counted and the slides with eggs untouched could be placed in new bottles with plenty of fresh food. The number of adults produced was very small (table 3) even with this careful treatment—in fact the percentage of adults hatched, compared with the number of eggs laid, is lower here (5 percent) than when the first method of treatment was employed (14 percent). Data from other sources show that the percentage of eggs which hatch in normal stock may vary from 20 to 90 percent of the number of eggs laid by any female.

It was formerly supposed that fused females were absolutely sterile. Besides proving this idea incorrect, an examination of table 2 revealed the fact that the three females which were mated with fused (*fu*) males gave no flies, while all four which were crossed with normal (*N*) males were slightly fertile. This is not correlated with the number of eggs laid, for one of the crosses of fused by fused yielded the largest number

TABLE 3
Showing the number of eggs laid and of flies hatched by fused females.
Glass slide method.

No. of culture	Parents ♀	♂	No. of eggs laid	No. of flies hatched
B	*fu*	6 *N*	56	1
D	"	"	7	0
E	"	"	35	0
G	"	4 "	23	0
H	"	6 "	21	2
M	"	3 "	12	2
Q	"	"	38+	3
R	"	"	39	5
Total			231	13

(88 eggs) observed from any pair in the group. The significance of the kind of male used was then investigated by making a more extended series of crosses. That it is of importance, is shown by the fact that the combination of fused with fused never produced any offspring although 49 such females were tested by being mated singly with two or more fused males, while, on the other hand, out of 62 bottles of fused females by wild (*N*) males, only 13 (or about 21 percent) were totally unproductive.

As table 4 shows, many of the 49 fertile females produced but one adult while others gave as high as 30 and 36 flies; but in the sum total of 560 offspring not one male was found.

That bar-eyed males were as effective as the normal when used as mates for fused, was proved by crossing them with twelve fused females and obtaining 263 flies. Two of the fused females, or 16 percent, were sterile. Again, all of the F_1 were females (table 5). Combined with the data listed in tables 2, 3 and 4, this gives 884 females and not a single male as the total number of offspring from 89 fused females, 76 percent of which (68 individuals) were fertile to at least a slight degree, provided they were mated with males from strains other than their own. As fused is recessive, the F_1 females were normal or bar.

The presence of a dominant factor in the hereditary complex of fused individuals does not alter the sterility of the females. Twenty-seven red-bar-eyed fused females, mated with red or eosin bar males gave no offspring although all carried the sex-linked dominant bar.

TABLE 4
Fused ♀ by wild ♂.

No. of culture	*N* ♀♀	*fu* ♂♂	No. of culture	*N* ♀♀	*fu* ♂♂
101	15		132	0	
102	32		133	8	
103	35		134	10	
104	30		135	1	
105	7		136	10	
106	29		137	25	
107	24		138	0	
108	2		139	10	
109	20		140	0	
110	8		141	15	
111	1		142	1	
112	2		143	3	
113	20		144	10	
114	11		145	3	
115	14		146	1	
116	5		147	5	
117	3		148	1	
118	2		149	15	
119	16		150	3	
120	1		151	36	
121	7		152	24	
122	1		153	16	
123	4		154	0	
124	1		155	21	
125	0		156	10	
126	0		157	4	
127	0		158	28	
128	0		160	0	
129	0		161	4	
130	0		183	0	
131	6		159	0	
Total			62	560	

b. *Search for a factor for sterility*

Although in these preliminary tests, designed to ascertain the degree of infertility of the fused stock, the results of using normal and bar males show that fused females are not to be classified as totally sterile, it is still evident that their performance is far below normal. Throughout the course of this investigation, it was constantly borne in mind that the disability here encountered might be due to the activity of a particular factor for sterility. If factors, distinct from those which

TABLE 5
Fused ♀ by bar ♂.

No. of culture	Bar ♀♀	Fused ♂♂	No. of culture	Bar ♀♀	Fused ♂♂
171	58		177	56	
172	20		178	25	
173	21		179	1	
174	10		180	2	
175	5		181	0	
176	65		182	0	
Total				263	

cause the mutations, control the productivity of the individual, we must suppose that the two kinds are closely associated in the germ cell if we wish to account for the constancy with which sterility accompanies the specific character. In other words, in order to cause such close linkage, the sterility factors must be situated in the same chromosomes as the mutant factors, probably not far removed from them. According to the theory of crossing over, it should be possible to separate the two kinds of factors if such exist, and obtain sterile races which do not show the mutation and *vice versa*. This theory is based upon the idea of the linear arrangement of factors in the chromosome threads and postulates that during some stage in the cell history, previous to maturation, when the maternal and paternal members of each pair of chromosomes twist about each other, they may permanently fuse at one or more points and when untwisting may break apart in such a manner that homologous sections of the two chromatin threads may be interchanged. If the point of fusion and breakage should occur in the interval between the loci of the factors under consideration, one of those factors would become incorporated in the opposite chromosome and the linkage between these factors would be broken. Thereafter, each one could affect the gamete to which it was distributed, free from the influence of the other.

In the absence of any definite knowledge as to the dominance or recessiveness of the sterility factor (the existence of such an entity being assumed) it becomes necessary to consider both possibilities. On the supposition that it is recessive, the fused female, with which we have been dealing, must have been homozygous for sterility, otherwise it would not have exhibited the character. A crossover which occurred in such an individual would be unavailing since, both chromosomes hav-

ing the sterility factor, this would simply be a trading of identical genes. It is in the normal female, heterozygous for fused (and for the sterility factor) that favorable conditions for effective crossing over exist. If the crossover did occur once, however, the fused female which received one chromosome free from the sterility gene, should be fertile,—at least to some extent, according to the degree of recessiveness, assignable to the sterility factor. In this case the attainment of an individual homozygous for the "not-sterile" factor would be unnecessary, for the female heterozygous for "not-sterile" should be able to found a fertile fused strain. However, the fact that the $\frac{N}{f_u}$ female does not seem to be quite as fertile as the pure normal individual, suggests that the influence of the fused gene or of the sterility factor accompanying it, is semi-dominant, at least in this particular chromosomal complex. If this be true, a fused female heterozygous for the sterility gene might be moderately productive. If the attainment of a fused female which would have a normal fertility depends upon its homozygosity in regard to the allelomorph of the sterility factor, an egg belonging to the crossover class must be fertilized by a sperm of similar chromosomal composition before the fertile fused race can be obtained. However, it is possible that if the sterility factor is dominant, or partly so, the crossover class may already exist and the "not-sterile" factor may be passing, undetected, through the fused stock. In this case the homozygous condition might be readily obtainable.

It is of interest to note a peculiarity connected with the method of producing the homozygous crossover class when dealing with a factor like fused. Ordinarily, in such an attempt, the heterozygous female could be mated with the recessive male and their F_1 offspring, exhibiting the recessive character, could be inbred on the chance that the crossover gametes might unite in the F_2. But where fused is concerned, outcrossing is always necessary. Fused by fused produces nothing at all. Fused females by foreign males give only females—and these are not fused. Inbreeding with the pure stock is impossible. Both kinds of individuals, both fused males and females must always be derived from the heterozygous female. In the latter type, however, the condition demanded for crossing over is always present.

Upon the conclusion of the experiments summarized in table 4, it was noted that certain of the pairs had produced quite a few individuals. As these pairs were regarded as the most likely progenitors of a fertile line,

should one be forthcoming, some of the heterozygous F_1 females from cultures 101, 103, 104, and 158 were mated with fused males and from the F_2 resulting, 63 pairs of fused brothers and sisters were made up. Here, just as in the preliminary experiment, the combination of fused with fused resulted in total sterility; showing that not one of the females had obtained by a previous crossover, either one "dose" of a dominant factor for not-sterile or two "doses" of a recessive factor for not-sterile, which would permit them to be fertile when tested by fused males. Including data given in the succeeding pages of this section, a total of 412 fused females was tested for fertility. Of these 170 were crossed with fused and the rest, 242, with wild or bar males. The highest number of offspring produced by any one individual was 86 which is far below the expectation from a fertile fly. On the assumption that the sterility factor is one unit from fused, one out of each one hundred eggs produced by a heterozygous female should belong to the crossover class of fused-fertile. If sterility is recessive, any fly formed from the crossover type of egg would indicate the fact by being fertile. If sterility is partially dominant, the homozygous recessive must be extracted before a completely fertile fused race is obtained. Fertilization of the crossover type of egg by any Y sperm gives a fused-fertile male and since there is no crossing over in this sex, the two genes remain associated during their residence in it. The union of an X sperm from this fused male with an egg of like constitution should accomplish the formation of the fertile fused female. The chances of such a meeting seem rather remote even among 412 trials, but it must be noted that should the crossover occur, the new combination of genes would remain united with as great tenacity as they formerly remained apart and, on the assumption that sterility is semi-dominant, might have travelled together for some time undetected in the stock.

c. *Production of but one sex by fused females*

One of the most striking points brought out by the work with fused, namely, that the females gave offspring belonging to but one sex, remained to be investigated. The suggestion that there might be some peculiarity inherent in the fused female which prevented her from producing males, made it pertinent to test the point. A solution of the problem was found by using non-disjunction.

Since the discovery of the occurrence of non-disjunction in Drosophila, by BRIDGES (1916), it has been known that females which possess an

extra sex chromosome, i.e., that have the unusual constitution XXY, can produce males of an exceptional kind which are formed by the union of a Y-bearing egg from the mother and an X-bearing sperm from the paternal parent, just the reverse of the ordinary process. If a fused XXY female were crossed with a normal male, we should expect the F_1 to give not only females but also the exceptional males unless the nature of the fused female prevented the formation of more than one sex. Accordingly a stock which carried the extra chromosome was so combined with the fused race that their F_2 would yield fused females, one-half of which would be expected to have three sex chromosomes, i.e., be XXY females.

One hundred and fifty-five of such individuals with eosin eyes were mated singly with red bar-eyed males. In the results from these tests the sterility was greater than in the crosses with the wild fly, as only 78 gave offspring. The expectation was that one-half or 39 of these should carry a Y chromosome and each of the 39 could throw the exceptional males. Five of these produced one to four exceptional males (table 6). These males had red and bar eyes and normal wings like the father, since their only X chromosome was obtained from the paternal parent which was red, bar and normal. This proves that it is possible for males to be produced from fused females. There were obtained also 744 bar-eyed females but no fused males, although the two classes are expected in equal numbers. Another exceptional class, that of the fused females, formed from an XX egg and a Y sperm, should also have appeared and in as large numbers as the bar-eyed males, but it was not found.

So far no fused female had ever given any offspring when crossed with males of her own kind, but in order to discover whether these XXY females might not behave differently and throw the exceptional classes, 81 fused females, half of which were expected to carry the extra Y, were mated with bar fused males (red-, white-, or eosin-eyed). No flies whatever were produced. Copulation was observed to occur in some cases and that it may be partially effective is demonstrated by the following observations.

In making counts of the eggs it was noticed that on the second day after having been deposited some of them have a small black spot at the anterior end, indicating that the chitinous larval mouth parts are formed. Removal of the chorion reveals the fact that segmentation of the body has taken place and cuticular hooks have been formed. Pressure upon the cover glass over the object may rupture the body wall and a

TABLE 6

Fused ♀♀, half of which should be XXY, by bar ♂♂.

No. of culture	Bar ♀♀	Bar ♂♂	No. of culture	Bar ♀♀	Bar ♂♂	No. of culture	Bar ♀♀	Bar ♂♂	No. of culture	Bar ♀♀	Bar ♂♂
B 1	57		L 6	6		S 34			S 73		
B 2	10		L 7			S 35	4		S 74	1	
B 3			L 8	8		S 36			S 75	1	
B 4	82	4	L 9	11		S 37			S 76	2	
B 5	6		L 10	11		S 38			S 77	1	
B 6	3		L 11			S 39	7	1	S 78	1	
B 7			S 1			S 40			S 79	7	
B 8			S 2	11		S 41			S 80	4	
B 9	24		S 3	19		S 42	6		S 81		
F 1			S 4			S 43	14		S 85		
F 2	2		S 5			S 44	4		S 86		
F 3			S 6	5		S 45			S 87	4	
F 4			S 7	4		S 46	1		S 88	3	
F 5			S 8			S 47	2		S 89		
K 1	5		S 9	1		S 48	2		C 1		
K 2	10		S 10	14		S 49	4		C 2		
K 3			S 11	9		S 50	5		C 3	2	
K 4			S 12	7		S 51			C 4	7	
K 6			S 13	4		S 52	2		C 5		
K 7	24		S 14	17		S 53	2		C 6	1	
K 8	1		S 15	36		S 54			C 7	3	
K 9	17		S 16	1		S 55	1		C 8		
J 1			S 17			S 56	1		C 9	21	
J 2			S 18			S 57	4		K 10		
J 3			S 19	3		S 58	2		K 11		
J 4	1		S 20	2		S 59	4		K 12	22	
J 5			S 21			S 60			K 13	5	
J 6			S 22	1		S 61			K 14		
J 7			S 23	19	1	S 62	3		K 15		
J 8			S 24	4		S 63			K 16		
J 9			S 25			S 64	1		K 17	36	
J 10	14		S 26	7		S 65			K 18		
J 11			S 27	32	2	S 66	4		K 19		
J 12			S 28	1		S 67			B 10		
J 13			S 29			S 68			B 11	25	
L 2			S 30	1		S 69			B 12		
L 3			S 31		1	S 70	12		B 13		
L 4	8		S 32	1		S 71	3		B 14		
L 5			S 33			S 72	1		B 15		
Total									155	744	9

tube, identified as the alimentary canal, may be squeezed out. After the second day the eggs begin to darken and show signs of disintegration.

In only two cases out of hundreds observed, have larvae hatched from the eggs and never have they been seen to pupate. The lethal influence, therefore, of the pair of fused genes may not have its full effect until the egg stages, or even the larval period, is reached.

d. *Discussion of peculiarities shown by fused*

In interpreting the foregoing results certain striking facts brought out by the experiment must be kept clearly in mind:

1. In spite of more than two hundred attempts, no cross of fused by fused has ever been successful although fused males are ordinarily fertile with females of other races and fused females may in some cases lay a good many eggs and may even produce adults when mated with males from other strains.

2. The sygotic combination of fused with fused is not fatal, as is shown by the fact that females heterozygous for fused, mated with fused males, will produce approximately as many fused males and females as they will yield normal males and females. Only a negligible difference between the two types is to be observed.

3. In the crosses of fused females with males of different kinds, only females (plus a few exceptional males) were produced, although as many as 1628 females have been obtained.

4. That the lack of males is not due to any inability of the fused females to form that particular sex, was demonstrated by using XXY females and producing, through non-disjunction, males of the exceptional class, having the characteristics of the paternal instead of the maternal parent.

An examination of the various types of crosses in which fused has been concerned, reveals the additional fact that success, or at least partial success, ensues only in those cases in which a normal allelomorph of fused is involved at some point in the scheme though not necessarily present in either of the gametes at the time when they unite to form the zygote. We are, therefore, inevitably led to the conclusion that the deleterious influence of the fused gene is of such a nature that it must—and can to some extent—be counteracted by that of its normal allelomorph in the other X chromosome, in order to insure the production of adults. Furthermore, we see that the normal X chromosome may exert its opposing influence at two points: (1) it may be effective as a member of the parental complex, i.e., in the oögonial cell before maturation, as in the instance of the heterozygous fused female where it effects the

formation of a large number (almost the normal number) of eggs, and these are capable of being fertilized by sperm even though the latter may carry the harmful fused gene; (2) when present in a gamete from a male, the normal X may have the ability to "redeem" an egg produced by a homozygous fused female and to enable it to develop normally.

One other consideration is necessary to complete an explanation for all the phenomena cited in the preceding paragraphs. This relates to the nature of the Y chromosome. The experiments indicate that it contains nothing which can counterbalance the injurious influence of the fused factor at either of the above-mentioned points. Not only does it lack this counterbalancing power itself, it cannot gain such power from association with a normal X. These considerations furnish an explanation of the facts found in a detailed analysis of the crosses made. Reference to figure 1 may make the subject clearer by giving a visual image of the types of chromosomal combinations obtained.

FORMULA 1.—*A normal female heterozygous for fused by a fused male.* All four of the expected classes, normal and fused, males and females, appear. The normal allelomorph of fused,—or of a factor for sterility lying near fused,—is carried in the normal X of the heterozygous female and is sufficient to cause the production of a large, though not quite the normal, number of eggs. It restores the eggs carrying the fused gene so that the two kinds are produced in approximately equal numbers and both are viable in combination with either the Y sperm or the sperm carrying the factor for fused in the X chromosome.

FORMULA 2:—*A fused female by a fused male.* No offspring have ever been given by this combination, which seems to be dependent on the fact that neither the eggs nor the sperm contain a normal allelomorph of fused in their chromosomal constitution. Sperm from a fused male are viable,—as witness the preceding cross; eggs from a fused female may be fertile, — note the succeeding cross; the combination of two fused gametes can also occur; the only difference to be observed between this case and the first is the absence of the normal allelomorph of fused in the maternal complex and to this circumstance must be imputed the failure to obtain any offspring.

FORMULA 3.—*A fused female by a normal male.* A single class of individuals appears,—the normal females. As we have seen, the egg production of the fused female is below normal but does not cease entirely. In type 2, the X sperm carrying fused proved unable to form a viable zygote in conjunction with an egg which had not been under

the influence of a normal X. That the X sperm from a normal or bar male possesses this ability, is demonstrated by the occasional appearance of normal-winged females in the F_1 of this cross. The non-appearance of males must be attributed to the inability of the Y sperm to serve a like function.

FORMULA 4.—*A fused female (XXY) by a bar-eyed or wild-type male.* Normal-winged females and males are given by this cross. Since maturation in this type of female differs from that of the normal, in several features, we may take up the analysis in more detail. Four kinds of eggs are expected to be formed: One with a single X chromosome which carries fused; one with two X chromosomes, each carrying fused; one possessing a fused X and a Y; one with a Y. The two kinds of sperm are the same as in the preceding cross,—one with a not-fused X, the other carrying a Y. The relationships here are similar to those in the case just discussed except for the addition of the Y in the maternal complex. As in the preceding case, only those individuals appear which are the result of a fertilization by an X sperm. This includes two types of females (XX and XXY) and the exceptional male (YX). No tests were made to distinguish whether the females actually were of different constitutions. Females containing XXX have never been found in any cross and are not expected here. The exceptional males, of which a small number were obtained, are formed by a union of an egg containing a Y with a sperm having a not-fused X. The greatest number of individuals given by any one female was 86 (table 6), which is slightly more than obtained from type 3 (tables 2, 3, 4, and 5), but by no means approaches the number produced by a female containing a normal X. This indicates that the Y chromosome cannot function like the normal X in the maternal complex in counteracting the effect of fused. It is evident that it possesses nothing which can overcome this harmful activity in the prematuration stages. The number of exceptional males expected is 4 percent of the total. Using the record of the most productive female for our computation we find that when 82 represents the number of F_1 females, the expectation for the exceptional males is 3.57. Actually 4 males appeared,—a very close approximation.

Fertilization by the Y sperm did not prove successful in a single case. The YY combination is not expected since it is supposed not to be viable, but that the X egg from a fused female is not fertilized by the Y, is explained by our assumption that the Y sperm does not con-

tain any property that can restore fused eggs nor can it acquire power so to do by association during spermatogenesis with a normal X.

FORMULA 5.—*A fused female (XXY) by a fused male.* No offspring whatever are produced. Eggs from such females and sperm from this type of male have proven viable in the preceding experiments. The particular gametic combinations expected are not impossible of realization under the proper conditions, yet no F_1 individuals are obtained. The explanation lies in the fact that the influence of the fused gene is not combated by the presence of its normal allelomorph either during oögenesis in the female or after the process of fertilization by the sperm.

RUDIMENTARY

a. The theories of prematuration and repugnance

The case of rudimentary wings, another sex-linked gene, located at 55.1 in the first chromosome, presents a less extreme example of the same sort of phenomenon as fused. The sterility of the females has been reported already by MORGAN (1912) and the theory of "prematuration" and "repugnance" suggested in connection with it. The first results indicated that the two cases possessed many similarities. MORGAN found that rudimentary flies when mated with rudimentary, were sterile; that rudimentary males were fertile with long-winged females; and that rudimentary females when fertilized by normal males gave a few offspring of both sexes. The only difference which destroys the parallel between the two cases, is in regard to the last point, for the F_1 of the fused female comprises only females while that of the rudimentary female represents males and females. It is true that most of the progeny were females, but a few males were found, in the ratio of about 1 : 300. A quotation will give MORGAN's conception of the element of prematuration.

"In the *heterozygous* female the egg has developed up to the time of the extrusion of the polar bodies under the influence of *M* (i.e., all the normal factors are present, at least in simplex) . . . Not until the time of polar-body formation is the factor *M* lost from half of the eggs, i.e., from those eggs that may produce rudimentary offspring. Hence the relatively large number of eggs that may be fertilized by the rudimentary-winged male. On the other hand in the *rudimentary* female the egg develops without the presence of the factor *M*. If the absence of this factor, in the prematuration development, makes the egg less fertilizable by any sperm, the difference in the behavior of the two kinds of females in question can be accounted for."

PARENTS OFFSPRING

(1) $\frac{+}{fu}$ (normal♀) × $\frac{fu}{Y}$ (fused♂) gives $\frac{+}{fu}$ (normal♀) $\frac{+}{Y}$ (normal♂) $\frac{fu}{fu}$ (fused♀) $\frac{fu}{Y}$ (fused♂)

(2) $\frac{fu}{fu}$ (fused♀) × $\frac{fu}{Y}$ (fused♂) gives $\frac{fu}{fu}$ (fused♀ absent) $\frac{fu}{Y}$ (fused♂ absent)

(3) $\frac{fu}{fu}$ (fused♀) × $\frac{+}{Y}$ (normal♂) gives $\frac{fu}{+}$ (normal♀) $\frac{fu}{Y}$ (fused♂ absent)

(4) $\frac{fu}{fu}Y$ (fused♀) × $\frac{+}{Y}$ (normal♂) gives

$\frac{fu}{+}$ (normal♀) $\frac{fuY}{+}$ (normal♀) $\frac{fufu}{+}$ (dies) $\frac{Y}{+}$ (normal♂ exception)

$\frac{fu}{Y}$ (fused♂ absent) $\frac{fuY}{Y}$ (fused♂ absent) $\frac{fufu}{Y}$ (fused♀ exception absent) $\frac{Y}{Y}$ (dies)

(5) $\frac{fu}{fu}Y$ (fused♀) × $\frac{fu}{Y}$ (fused♂) gives

$\frac{fu}{fu}$ (fused♀ absent) $\frac{fuY}{fu}$ (fused♀ absent) $\frac{fufu}{fu}$ (dies) $\frac{Y}{fu}$ (fused♂ exception absent)

$\frac{fu}{Y}$ (fused♂ absent) $\frac{fuY}{Y}$ (fused♂ absent) $\frac{fufu}{Y}$ (fused♀ exception absent) $\frac{Y}{Y}$ (dies)

FIGURE 1

FIGURE 1.—Formulae of crosses testing fused flies. Each symbol represents a separate sex chromosome. Fused X = fu; not-fused X = +.

By the theory of "repugnance," MORGAN endeavored to explain the fact that in crosses between a female, heterozygous for rudimentary, and a normal male, the rudimentary class fell below expectation. He suggested that there was a repugnance between the rudimentary-forming gametes, that is, between the gametes which lack *M*. (In

referring to the original paper, it must be kept in mind that the terminology of the presence and absence theory was used and where the statement is made that "sperm lacking the character *M*, fertilizes with difficulty eggs lacking that same character," the Y sperm is considered to be "without" the above-mentioned *M* in the same way that the rudimentary-bearing egg is "without" it.) Statistics which prompted this explanation were given for a number of cases. In the first, there were 2061 miniature males and 479 rudimentary miniature males, a ratio of about 4.3 : 1 where a 1 : 1 ratio is expected. In the second, there were 342 miniature males to 98 rudimentary miniature, a ratio of 3.5 : 1. Several others gave ratios of 2 : 1 and 3 : 1.

MORGAN calls attention to the fact that his results represent an "improvement in the viability" of the rudimentary stock since its discovery. At first it had yielded 115 rudimentary males to 4773 (calculated) normal males, when equality was expected. Additional data published several years later (MORGAN and TICE 1914) showed that the disparity between the two classes of flies was due to unfavorable culture conditions rather than to a principle of repugnance. When overcrowding in the culture bottles was avoided, little repugnance was shown between any gametes forming the rudimentary classes either male or female. My observations on five matings of heterozygous females by rudimentary males, in which comparatively small numbers were involved, agreed with those of MORGAN and TICE. The total for five bottles gave 459 normal to 406 rudimentary males and 425 normal to 345 rudimentary females where equality is expected (table 7).

In the case of fused also, the principle of "repugnance" may be said to be inapplicable as is shown by the results given in table 1 from a similar cross where equality of the four classes is expected.

TABLE 7

Females heterozygous for rudimentary, by rudimentary males.

No. of culture	N♀♀	*r*♀♀	N♂♂	*r*♂♂	Total
900	124	80	130	136	470
901	102	90	108	88	388
902	96	95	119	91	401
903	69	40	56	45	210
904	34	40	46	46	166
Total	425	345	459	406	1635

In a later paper published by MORGAN (1915) the data for certain successful matings between rudimentary males and rudimentary females seemed to cast doubt upon the hypothesis of prematuration. The same type of cross in the fused stock, i.e., fused females by fused males, has never been attended with success except so far as to produce one or two larvae. That rudimentary by rudimentary may occasionally give offspring might be regarded as merely indicating that the injurious influence of the rudimentary gene is not so far-reaching as that of the gene for fused, without contradicting the general principle that particular genes may affect to a greater or lesser degree, the formation of the egg in the stages prior to the maturation divisions.

The principle of "restoration" outlined in the case of fused, is amply illustrated in the following experiment. The addition of a Y chromosome to the ordinary complex of the rudimentary female has an effect upon the formation of future classes similar to that obtained in the case of the non-disjunctional fused female, as the following data show. Sixty-five XXY rudimentary females (each X having a rudimentary gene) were mated separately to bar males (table 8). Fifteen bottles were sterile. The remaining fifty bottles gave 647 bar females, 2 rudimentary females, 41 bar males, and 52 rudimentary males. The four expected classes are represented by the four types obtained. An analysis of the cross may make this clearer. As explained in the case of the similar experiment with fused the XXY rudimentary female parent possesses three sex chromosomes, so that synapsis may occur in two ways (figure 2). There may be an XX synapsis and the Y may either pass out into the polar body or remain in the egg, thus forming eggs containing one X, or an X and a Y. In the second kind of synapsis the X is paired with the Y and the other X may pass out of, or stay in, the egg, forming gametes of the constitutions XX or simply Y as well as the XY and X classes. From analogy with other data, we assume that the XY eggs should comprise 46 percent of the total, the X eggs 46 percent, and the XX plus the Y 8 percent. All of these eggs should normally be capable of fertilization by either the X- or the Y-bearing sperm with two exceptions: the Y eggs fertilized by a Y sperm and the XX eggs by the X sperm, do not produce viable zygotes. Successful fertilizations by the Y sperm produce the recessive rudimentary classes.

In analyzing this cross, we find a second indication that the influence towards sterility of the rudimentary gene is not as great as that of fused, both because rudimentary males are obtained and because 2 rudimentary

TABLE 8

Red rudimentary XXY females by bar males.

No. of culture	Rud.	Bar ♀♀	Bar ♂♂	Rud. ♂♂	No. of culture	Rud. ♀♀	Bar ♀♀	Bar ♂♂	Rud. ♂♂
575					610		3		
576		50			611		7		
577		53	2	2	612				
578		8			613		11	1	
579		4		1	614		7		
580					615		12		1
581					616		26	2	3
582		13	1		617		3		
583					618		11		
584		16		2	619		1		
585					620		18	6	
586					621		1		
587					622		4		
588		35		3	623		5		1
589		26	3	6	624		18		1
590					625		17	1	4
591		2	1		626		4		
592					627		7		
593					628		35	2	1
594					629		6	3	1
595		13		1	630		6		
596					631		6	1	
597		9		1	632		43	9	14
598					633		6	2	1
601		1			634		9		
602		1			635		43		2
603		3		1	636		27	1	1
604					637		25		2
605		1			638	2	12	2	
606			1		639		1		
607		10			640		1		
608		3	2		641		16	1	1
609		8		2					
Total					65	2	647	41	52

exceptional females appear. The two corresponding classes have never as yet been obtained from any fused female. Here, the number of each is, however, below expectation. The rudimentary males should equal the bar females instead of being 52 to 647. This comparison, therefore, may be taken as a measure of the "restoring" power of the normal allelomorph of rudimentary. The X- and XY eggs which are produced by the rudimentary parent, all carry rudimentary and are of the same sort, no matter whether they are destined to be fertilized by an X- or a Y-bearing sperm.

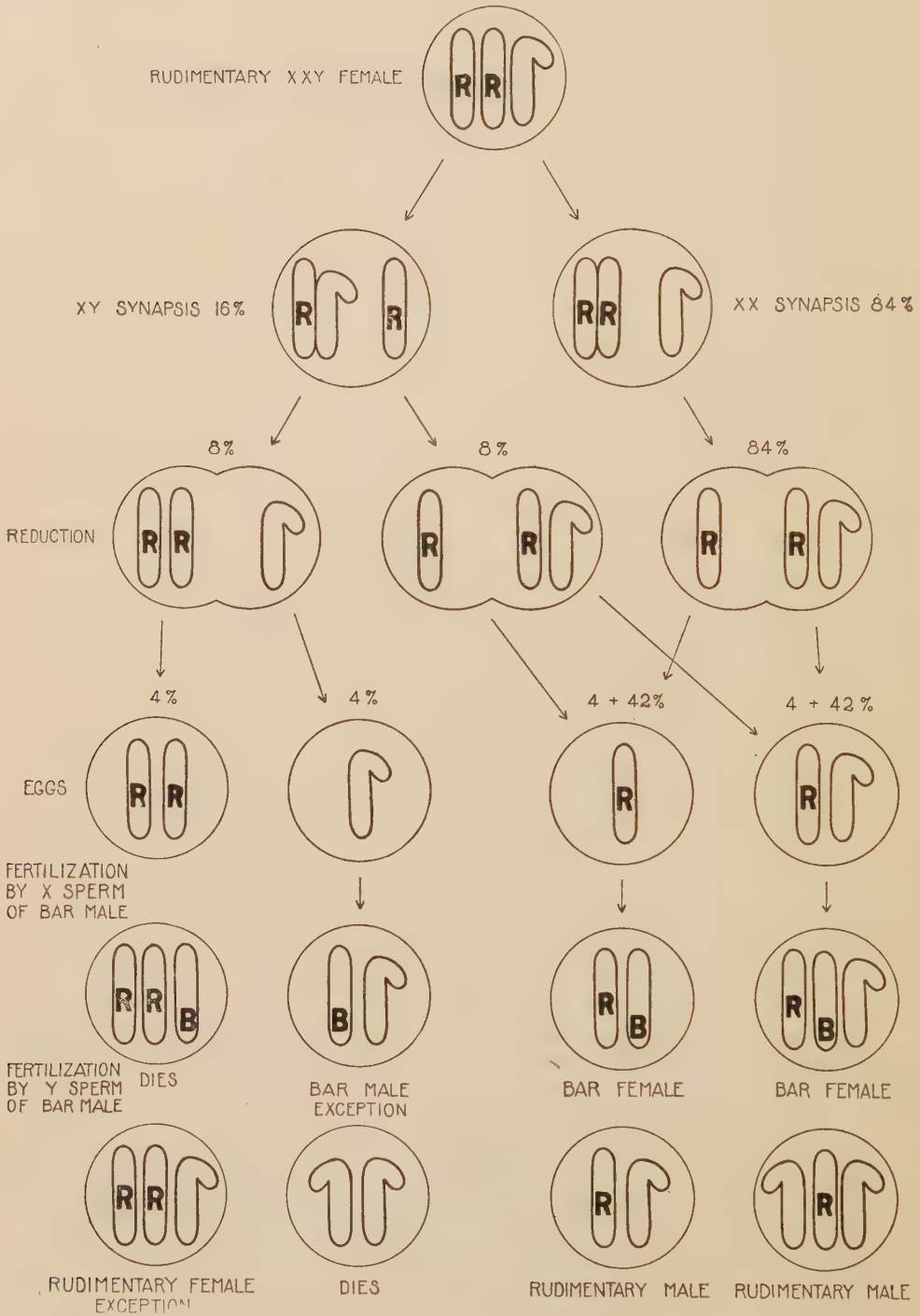

FIGURE 2.—Diagram showing the chromosomal constitution of an XXY rudimentary female, the two types of synapsis which occur in such an individual, reduction, the four possible kinds of eggs and the zygotes which would result from the fertilization of these eggs by sperm from a bar male. The X chromosome is represented as a straight rod and the Y as J-shaped. R = rudimentary, B = bar.

The zygotes formed from the two types of fertilization differ greatly in viability. The male zygotes are markedly fewer in number than the female. The Y chromosome which is brought in by the male-producing sperm, contains nothing, *ex hypothesi*, which can alter the viability of the egg. It does not change the percentage of sterility. The number of rudimentary males resulting from the XY and XYY zygotes is one-half the number which represents the degree of viability of the rudimentary egg when unaided by any influence from the sperm. We must suppose that a larger number of females than males is produced because the X-bearing sperm, which form the females, contain the normal allelomorph of rudimentary and have the ability in some degree to counteract the prematuration effect of the rudimentary gene upon the egg and to restore the normal process of development. The number of bar females produced compared with the number of rudimentary males, i.e., 647:52, is a measure of the "restoring" power of the normal allelomorph carried by the X sperm and operating after fertilization.

The two exceptional classes are the bar males and the rudimentary females which should be equal to each other and together should comprise 8 percent of the total progeny. The eggs which form these two classes are the result of an XY synapsis which is followed by the inclusion of the second X in the same cell with the first X, thus giving two types of eggs, —one with two sex chromosomes, XX, and one with but one sex chromosome, the Y. The first type of egg fertilized by a Y sperm, makes a rudimentary female; the second type fertilized by an X sperm, makes a bar male. This type of synapsis occurs in 16 percent of the total but as one-half of the possible zygotes derived from it are not viable, the individuals of either exceptional class are 4 percent of the total number of fertilizations by either an X sperm or a Y sperm. For example, the bar males compared to the bar females should be as 4 percent is to 92 percent, and the rudimentary females to rudimentary males should also be as 4 to 92. The total output of eggs is far below normal, probably due, as has been previously suggested, to the adverse influence upon development, of the rudimentary gene. There is no reason to suppose that the ratio of the various classes would be changed in any way from that expected in any cross involving non-disjunction. A comparison of the number of bar males to the bar females obtained, is perfectly legitimate in that the principles of both prematuration and restoration are at work in the type of union which results in the formation of both classes. A computation based on such a comparison gives 28 as the number of

bar males expected. Actually there are 41. The fact that the percentage of non-disjunction is known to be variable may account for this disparity.

In the type of reproduction which gives the rudimentary males and females, only one principle is in action—that of prematuration. As the sperm concerned in these fertilizations carries a Y as its only sex chromosome and does not possess any element to combat the injury of the prematuration influence, the principle of "restoration" is absent. Therefore, fewer rudimentary females are expected than bar males, although under normal circumstances the classes should be equal. As previously stated, the rudimentary females should be to the rudimentary males as 41 is to 92. The numbers actually obtained (2 to 52) are in quite close agreement with expectation.

b. *Search for a factor for sterility*

In continuation of the search, begun in the case of fused, for a gene governing fertility and distinct from that of the mutation involved, it was deemed advisable to test some of the individuals of the previous experiment to see whether a fertile race of rudimentary flies could be obtained. The rudimentary females from the above cross died before they were tested in regard to fertility. The other females were long-winged and had to be mated with rudimentary males in order to produce rudimentary offspring which in turn could be tested for fertility. This was done and 100 of the rudimentary females thus obtained were mated in mass cultures with their rudimentary brothers and 75 were crossed with bar males from stock (table 9). If, by means of a crossover in the heterozygous female the rudimentary gene had become separated from a sterility gene, some of these females tested might reflect this event by producing a large number of offspring. However, none of them gave as many individuals as the grandparents, one of which had yielded as many as 66 flies (table 8).

c. *Additional mutations to rudimentary*

Rudimentary has appeared twice in other stocks kept in the laboratory. One of these mutations to rudimentary, found by Mr. D. E. Lancefield (1918), was tested by crossing five rudimentary females with bar males. No offspring whatever resulted, indicating that this second mutation to rudimentary was also accompanied with sterility. Whether a previous mutation to sterility had occurred at a point adjacent to the rudimentary locus, and was made apparent only by the modifying action of the rudimentary gene is a question not settled by the evidence.

TABLE 9

Rudimentary ♀♀ by rudimentary or bar ♂♂.

No. of culture	Parents ♀♀	♂♂	F_1 ♀♀	F_1 ♂♂	Total
702	11	× 6 rud.	1	2	3
703	6	3 "	3	1	4
704	10	5 "			
705	4	12 "	3	10	13
706	3	4 "			
708	11	6 " +1B′	10	8	18
709	4	2 "	3	8	11
710	3	3 "			
711	9	7 "	18	±5	±23
713	10	10 "	6	7	13
719	12	7 "	2	5	7
714	10	10 "	6	5	11
720	7	7 "	2		2
715	33	13 B′	20	3	23
716	18	7 "	28	7	35
717	22	33 "	13	8	21
718	2	2 "			
Total	175		115	69	184

Dr. A. H. STURTEVANT found rudimentary a third time; in this instance, in a strain of black purple curved flies. A cross between this newest rudimentary and a normal female heterozygous for the original rudimentary gene gave normal and also rudimentary males and females. Had the new mutation been dependent upon a gene different from the first, their F_1 should give only normal offspring, as each parent would carry in its chromosomal constitution the normal allelomorph for the opposite factor. Since the recessive class of rudimentary females appeared in the F_1 we must conclude that each parent contributed the same gene and that the third mutation of rudimentary was therefore identical with the first.

The STURTEVANT stock was then tested for the possible occurrence of a fertile individual. Of 72 females, crossed with bar males, none yielded more than 57 offspring and 45 (about 62 percent) did not reproduce at all (table 10). The sterility here seems to be more pronounced than in the first rudimentary stock but this is perhaps to be referred to temperature conditions rather than any real difference in the nature of the gene. This strain was kept up by mating some of the heterozygous females with rudimentary males and the tests were continued upon their offspring. Twenty-seven of the rudimentary daughters were mated with one or

TABLE 10

Rudimentary ♀♀ by bar ♂♂ (STURTEVANT stock).

No. of culture	Bar ♀♀	Rud. ♂♂	Total	No. of culture	Bar ♀♀	Rud. ♂♂	Total
1001				1037			
1002				1038			
1003				1039		1	1
1004				1040			
1005				1041			
1006	1		1	1042	11	2	13
1007				1043	2		2
1008				1053	5		5
1009	1		1	1054	+		+
1010				2002			
1011				2001	+		+
1012	1		1	2003			
1013	5		5	2004			
1014	2		2	2005	1		1
1015				2006	3		3
1016				2007			
1017				2008	1		1
1018				2009			
1019				2010	1		1
1020	1		1	2011			
1021	2		2	2012			
1022				2013			
1023				2014	2		2
1024				2015			
1025				2016			
1026	1		1	2017			
1027				2018			
1028				2019	1		1
1029				2022			
1030				2023	2		2
1031				2024			
1032				2025	2		2
1033				2026			
1034		2	2	2028	+		+
1035	2		2	3221	31	26	57
1036				3225	+		+

more rudimentary brothers (table 11) and 14 of these bottles gave offspring. Nine rudimentary females, out-crossed with bar males, all reproduced, but none of either of these groups gave more than a dozen flies (culture No. 4095 to 4103, inclusive). In addition 94 rudimentary females were crossed with bar males (Nos. 4020 to 4105 and 4152 to 4160). An exact record of their performance was not kept but a large number of offspring was not given by any one of them. Among the 202 females of the STURTEVANT strain, tested for sterility, a completely fertile individual was not found.

TABLE II

Rudimentary ♀♀ by rudimentary ♂♂ (STURTEVANT *stock*).

No. of culture	♂ and ♀ offspring	No. of culture	♂ and ♀ offspring	No. of culture	♂ and ♀ offspring
3251	2	3240	3	3248	1
3252	1	3243	2	3249	1
3236	2	3245	2+	3250	4
3237	2	3246	3	3262	1
3239	3	3247	8		

Thirteen other cultures gave no offspring.

MORULA

"Morula" eye is dependent upon a gene located at 106.3 in the second chromosome. It is characterized by great irregularity in size and protuberance of the ommatidia. In addition, the reduction of the bristles on the thorax is of such regular occurrence that their condition may be taken as diagnostic of morula when the eye disturbances are scarcely detectable.

The females lay only a few eggs. Five, which were observed for ten days, gave respectively 0, 0, 4, 9, and 4 eggs, none of which hatched. The males are probably perfectly fertile as no difficulty is experienced in keeping up stock by means of crossing them with the heterozygous female.

As the female shows a very high degree of sterility, it is evident that the few eggs which are laid are seldom capable of normal development. Three hundred morula females were mated with morula males in small mass cultures. No offspring were obtained. In order to discover whether males, other than her own kind, were more effective as mates, which had been observed to be the case with fused, 107 bottles were made up, each containing one female and 1 to 3 red bar or eosin crimson males. With sterile stocks difficulty is often experienced with the growth of mold upon the banana used as food, which may prevent the development of flies that might come through if it were not for this adverse condition. In this experiment the expedient was tried of inoculating the 107 bottles with the eggs of yellow flies so that the larvae of the more viable stock might churn up the food and by their activity destroy the mold. The adults of this stock could easily be distinguished from any gray morula flies which might hatch at the same time. Even with this precaution, the percentage of fertility was not materially increased

as only one of the 107 females gave offspring. She produced but two individuals, both normal-eyed females. Crossing these heterozygous females with morula males gave normal and morula males and females. Some of the males had yellow bodies, showing that the original morula grandparent had been fertilized by one of the yellow flies with which the bottle had been inoculated instead of by a red bar or eosin crimson male. Probably, no particular significance attaches to this fact. Eighty-eight of the F_2 morula females were mated with morula brothers, either yellow or gray-bodied, but none of them reproduced.

These figures indicate the very high rate of sterility among morula females as only one individual in the 500 tested gave offspring. The same considerations in regard to the possibility of sterility being dependent upon a separate gene, apply here as in the case of fused or rudimentary. Each one of these females was derived from a heterozygous parent which fulfilled the conditions necessary for crossing over. Had a chromosome containing morula, but free from the factor for sterility, been made up during this process, the occurrence would have been detected in the individual formed from it, provided sterility is recessive. Even if it is semi-dominant, either by itself or as a modifier of morula, we might hope that the homozygous recessive, i.e., for fertility, might have been formed during so many tests. That no morula female evinced normal fertility must be taken to indicate that sterility is dependent upon a gene lying exceedingly close to morula or that it is one of the manifold effects of morula itself.

REDUCED BRISTLE

Among the mutant stocks which proved sterile was one named "reduced bristle" because of the fact that the bristles on the thorax are reduced either in size or number. Stock is kept up by mating the heterozygous female with reduced-bristle males as the reduced-bristle females showed a high degree of unproductivity.

Observations on the egg-laying capacity were made in but two cases. The first female laid no eggs at all during a period of 9 days and the second laid 77 in 11 days. None of these hatched.

Of 70 reduced-bristle females tested for fertility with Australian (wild type), bar, California (wild-type) or eosin crimson males, only three gave offspring, producing 1, 37 and 16 flies, respectively. The difficulty of obtaining males which was evident in the fused and rudimentary stocks was conspicuously absent here as No. 53 gave almost half again as many males as females, i.e., 23 to 14, and No. 54, 7 males to 9 females.

This fact is in no way a contradiction of our theory of "prematuration and restoration," but is rather a confirmation of it and is explicable on the ground that here we are not dealing with a sex-linked factor.

The homozygous reduced-bristle females, as we have seen, lay very few eggs. We assume this is owing to the fact that the harmful influence of the pair of reduced-bristle genes (or of the sterility genes lying adjacent to them) is unchecked by any normal allelomorph in the prematuration stages of oögenesis. Some of these eggs, however, may be fertilized and brought to complete development by the redeeming influence of the gamete from the male. Since reduced bristle is not sex-linked but lies in the second chromosome its normal allelomorph is contained in duplex in all males used in the experiment. Therefore, all sperm, whether X- or Y-bearing, also carry an autosome which possesses an allelomorph to reduced bristle (or sterility) and have the power to counteract, in some measure, the injurious reactions started in the egg by reduced bristle, and produce viable zygotes. Since both male- and female-forming sperm have this capacity, both sexes are represented in the F_1. As reduced bristle is recessive, the F_1 flies appear normal.

The three productive lines were continued by out-crossing some of the bar-eyed daughters heterozygous for reduced bristle, to reduced-bristle males from stock and the reduced-bristle F_2 females obtained in this way were tested to see whether they were fertile. Among the F_2, of line 53, 16 females were mated in small mass cultures with males of their own kind but produced no flies. Twelve females from line 54 and 25 from line 52 gave similar results, showing that in a total of 125 females, not one possessed the reduced-bristle factor free from a factor for sterility, although three of them were not totally sterile.

DIBRO

The name "dibro" is an abbreviation of the three principal characteristics: dichaete, beaded and rough, exhibited by one of the strains which proved sterile. Besides having reduced bristles, beaded wings, and irregular eye facets, the abdomen is noticeably shorter and smaller in circumference than in the normal fly. The stock died out before the gene was located in any chromosome. BRIDGES, who found this mutation, reports the appearance of only one male, which was sterile, and in my cultures the four males which appeared were so weak that they lived only a short time.

The ovaries of the dibro female are small and the eggs remain im-

mature. Observation of twelve females, mated with bar or dibro males, for periods of from 9 to 16 days showed that no eggs had been laid.

Three females mated with bar males produced no offspring during the five or six days which they lived. Five more, mated with bar males, also showed sterility. The mutation appeared in eosin-eyed cultures and stock is maintained by making a large number of pair matings from the bottle which threw dibro. The appearance of dibro from any one pair shows that each one of the parents must have carried the gene in question and indicates the proper line from which to continue the stock.

DWARF

Aside from the fact that they are often lighter in color than the normal stock, dwarf flies show few differences from the wild type except that implied by their name. Frequently they are smaller than the wild fly, being approximately one-half the normal size but the range of variability is very great and some individuals may overlap the normal. The eggs also are smaller than those of the wild fly. The long axis is considerably shorter giving the egg a chubby appearance. Not all the females lay eggs. Of the eight females mated with either bar, dwarf, or wild males, and placed under observation for various periods of time, three produced no eggs, the other five gave 143, 50, 143, and 108, respectively (table 12). In the batch of 143 eggs, 54 of them dried up and therefore must not be included in the fertility percentage. From the total of 291 eggs, all from crosses in which bar or normal males had been used, only seven flies hatched. They were either bar or normal females. It is to be noted that no males appeared, but this fact is probably not to be regarded as significant in view of the small numbers involved.

TABLE 12

Showing number of eggs laid by dwarf females and of flies hatched therefrom.

No. of culture	Parents ♀	Parents ♂	No. of days observed	Eggs laid	Flies produced
1	*dw*	*B'*	6	0	0
2	"	*dw*	12	0	0
3	"	*N*	12	89+54	4
4	"	*B'*	14	50	1
5	"	"	9	43	0
6	"	"	14	1	0
7	"	"	14	108	2
8	"	"	11	0	0
Total				345	7

In a previous experiment, in which no counts of the eggs were made, the fertility of 31 adults was tested and found to be zero. Two of the heterozygous females obtained from the group of 108 eggs in the egg-count experiment were mated to dwarf males and gave 160 normal flies to 21 dwarf females and 25 dwarf males. Twenty maroon dwarf females from this cross and 4 from another F_2 group, were placed with bar or dwarf males, but did not yield a single individual. To sum up, 3 out of 63 females proved slightly fertile.

CLEFT

Cleft (denoting a peculiarity in venation) is a sex-linked recessive (locus 65). Only males of this mutant type have appeared. Since they are sterile, it has not been possible as yet to obtain any cleft females. Stock is kept up by mating the daughters of any female which has thrown cleft.

Examination shows that the testis is usually misshapen, often with the parts more or less fused, but containing live sperm. Copulation has not been seen to take place. Seventy-four males were put in separate bottles to which were added females from California (wild type), Australia (wild-type), bar or eosin crimson strains and, in two cases, normal females heterozygous for morula. These bottles had previously been inseminated with larvae from yellow stock in order to keep the food in good condition. Four of them gave a few offspring but until tested it was not certain whether their paternal parent had been a cleft male or a yellow male that had chanced to hatch early. When the female offspring mated, the kind of males they produced would indicate their ancestry, for both cleft and yellow are sex-linked and would therefore appear in the F_1. The results of the test of the daughters gave yellow males in every case, but no cleft, proving the paternal parent to have been yellow.

In regard to the possibility of separating an (assumed) sterility factor from that of cleft it is to be noted that the separation could not take place in the male, since crossing over does not occur in the male of Drosophila. In the heterozygous female from which cleft males were derived, however, fertile sons might be produced. In the seventy-four tests made such an individual was not discovered.

GENERAL DISCUSSION

The words fertility and sterility are blanket terms, covering a wide variety of conditions and descriptive of the degree of activity of a physiological function. The terms do not stand for an allelomorphic

pair of genes. We may suppose that every species has achieved, during the course of evolution, a characteristic mechanism for propagating its own kind that has sufficed to effect its survival. This process of normal reproduction entails numerous complexities, the omission or dislocation of any one of which might bring about sterility. In many cases the sterility might be accidental and not transmissible from parent to offspring. On the other hand, it is evident that a large number of either physiological or morphological differences affecting the formation or growth of the germ cells, or the mechanism connected with their liberation or fertilization, might upset the normal function of reproduction and be dependent upon some chemico-physical basis in the germinal constitution which would be passed on from one generation to the next as a true hereditary factor. Any departure from the normal may alter the balance of the reaction either towards an improvement over the condition ordinarily maintained or towards a less favorable one. If "fertility" expresses the normal condition, definitions may readily be found for such terms as "high fertility," "partial sterility," etc., the normal always being retained as the standard of comparison. In a case like fused, the two conditions found in the female of complete sterility with its own males and partial fertility with males from other strains are probably both the manifestations of a single determining cause in the germ cell. Here, the number of individuals which reproduce and also the number of offspring from each individual, is affected.

At present, it is not possible to state definitely whether the determining causes for the cases reported in this paper, consist of separate genes which have sterility as their principal somatic expression or whether they are an integral part of the genes responsible for the associated character, but the latter seems probable. It is evident that we are dealing with the activity of some factor and not concerned with inbreeding, with heterozygosity or the proportion of dominants present, all of which conceptions have been proposed as explanatory of various instances of sterility or fertility.

The sterility of three of the mutants reported here, rudimentary, fused, and reduced bristle, is evidently of the same type and may be elucidated by reference to the same theory. Morula, dwarf, cleft and dibro present no contradictory features. The fact that cleft is sterile in the male sex is readily harmonized with our theory on the assumption that the injurious influence of the cleft gene in the heterozygous female is so severe that it extends to the males produced by her, thus making them incapable of producing offspring.

The most clear-cut case is that of "fused" and a detailed inquiry re-

veals something of the mode of operation of the sterility connected with it. We have seen that the influence that causes the sterility is progressive, not acting with finality upon all of the gametes at any one point, but making itself felt throughout development. At two points in the process it is capable of being counteracted by its normal allelomorph. The harmful reactions (or inhibition of proper activities) set up by the gene, operate during oögenesis, in the stages previous to maturation and result in the production of a very small number of eggs unless their effect is checked by the presence of the normal allelomorph in the maternal chromosomal complex. In case the normal allelomorph is absent during prematuration, the few eggs laid perish unless the sperm which fertilize them bring in the normal allelomorph and restore to a certain percentage of them the usual course of development. The result will be that some of the females will produce a small number of offspring. When a sex-linked factor, such as fused, is concerned, the allelomorph is contained only in the X chromosome, i.e., the female-forming type of sperm, and therefore the F_1 from a fused female results in females only. In the case of a non-sex-linked factor, like reduced bristles, either sperm may carry the normal allelomorph in the autosome so that both sexes are comprised in the filial generation. On the same principle, morula and dwarf would be expected to yield some F_1 males if larger numbers were involved.

Rudimentary does not afford such a clear-cut case as fused but analysis of the data shows that the same principles are at work as in fused although the harmfulness of the latter gene is greater than that of rudimentary. A few rudimentary males may be formed when rudimentary females are fertilized by normal males and the cross of rudimentary by rudimentary is sometimes partially successful. But that the difference between rudimentary and fused is one of degree rather than of kind justifies us in including them in the same general category.

An interesting comparison, though not an exact parallel, in regard to the possibility of a prematuration influence, is afforded by the case of self-sterility in Nicotiana, reported by East and Park (1917). The non-occurrence of self-fertilization is explained by reference to the fact that male and female gametes have developed in the same individual and therefore under the influence of identical factors. Incompatible crosses are due to "the likeness of the parents in the effective hereditary factors postulated." The male gametes themselves may be of different kinds yet none can fertilize eggs from the same—or a similar—source as themselves. The prematuration influence in these plants appears to

be dependent upon the presence in the parental complex of a factor for self-sterility together with an unknown number of additional genes instead of being narrowed down as in the case of fused to the influence of one particular factor. The analogy of the cases does not extend to the conception that the identity of parental constitutions, regardless of the gametic type, is responsible for the phenomena observed but merely that the sterility is determined by activities in the diploid cell prior to maturation.

The experiments with fused have demonstrated that the Y chromosome is beyond the influence of the factors in the X chromosome. Recent considerations on the structure and formation of the Y chromosome, published by MULLER (1918), indicate that it is an accumulation of recessives, the product of degenerative changes in the factors. On this basis, it would not be expected to contain a gene in the locus corresponding to that of fused in the X chromosome that would be even remotely related to it. The behavior of the Y chromosome here, showing that it cannot be influenced by fused nor counteract the influence of fused upon other parts of the cell, is in accord with MULLER'S work.

While an exhaustive search for sterility factors is still to be completed, certain facts concerning the nature of the element at work have been pointed out, whether these peculiarities are to be regarded as dependent upon the presence of a distinct entity, the sterility factor—or factors—or whether they are but additional manifestations of the mutant genes themselves. All of the cases investigated appear to belong to the same type, the most clearly defined instance being that of fused. The corroborative evidence furnished by the latter suffices to rehabilitate the discarded theory of prematuration and although the principle of repugnance has not been substantiated an additional one has been suggested, namely, that of restoration.

SUMMARY

1. Males of the following races are fertile: fused, rudimentary, morula, reduced bristle, and dwarf. Cleft males are sterile.

2. Of the few individuals of dibro which were tested, both males and females were sterile.

3. When mated with males of their *own* race, fused and morula females are sterile, rudimentary females are partially so.

4. When mated with males of *other* races, a certain percentage of morula, dwarf, reduced bristle, rudimentary or fused females may give a few offspring; in a few cases morula and dwarf produced a very small number of individuals, all females; occasionally reduced bristle may

reproduce, giving a small number of each sex; rudimentary may yield a larger number of individuals, with females predominating; from 413 fused females, 1628 females and no males (except a few produced through non-disjunction) have been obtained.

5. In no case has it been possible to isolate a factor for sterility independent of the mutant factor itself. In each case the sterility shown is, therefore, probably one of the manifold effects of the gene responsible for the mutation.

6. The sterility involved decreases not only the number of fertile individuals in each race but also the number of offspring produced by each fertile female. However, the harmful effect of the mutant gene upon the egg may be counteracted at two points in the history of the gamete: (1) in the prematuration stages of oögenesis, that is, if the female is heterozygous for the mutant gene, a larger number of individuals will be produced than if she is homozygous; (2) if, at the time of fertilization, the sperm brings in the normal allelomorph, even though the female is homozygous and the number of eggs consequently reduced, a larger number of offspring will result than when the sperm carries the mutant gene. The Y chromosome has no corrective influence.

7. No "repugnance" has been observed between the gametes forming the recessive mutant classes.

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